Plant Biotechnology 23, 129–135 (2006)

Original Paper

Reducing gene flow from pollen dispersal of genetically modified plants in special screened greenhouses

Shin Watanabe, Tsunenori Sano, Hiroshi Kamada, Hiroshi Ezura*

Gene Research Center, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan
* E-mail: ezura@gene.tsukuba.ac.jp Tel & Fax: +81-29-853-7263

Received October 4, 2005; accepted December 22, 2005 (Edited by T. Nakamura)

Abstract The evaluation of genetically modified (GM) plants under special screened greenhouse conditions is the first step of assessing the risks of pollen dispersal from GM plants. To obtain fundamental information on the effective use of special screened greenhouses, we examined the pollen grain sizes of the major plants used for transgenic studies in Japan to estimate the effective fine-mesh size for covering the openings of special screened greenhouses to reduce pollen dispersal. Second, we examined the potential of small insects as possible pollen vectors. Finally, we investigated the relationship between fine-mesh size and air temperature in a covered space using a model of a special screened greenhouse. Although using smaller mesh sizes for covering the openings was clearly more effective in reducing pollen dispersal, the smaller mesh raised the air temperature substantially and resulted in poorer growing conditions for plants. We discuss this dilemma and suggest conclusions based on our research.

Key words: Cartagena protocol, genetically modified plants (GM plants), gene flow, pollen dispersal, physical containment.

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity took effect on 11 September 2003. Over 50 countries agreed through this protocol to endeavor to ensure that any living modified organism, whether imported or locally developed, undergoes an appropriate period of observation that is commensurate with its life cycle or generation time before it is put to its intended use and that a risk assessment is to be conducted prior to the first release of a living modified organism (UNEP 2004). Each country has the right to set standards for contained use within its jurisdiction. Additionally, countries are obligated to implement some measure of containment for a living modified organism during the period of risk assessment in each domestic research institution (UNEP 2004).

When the target living modified organism is a plant species, special measures are required because most higher plant species produce highly mobile transgene vectors, such as pollen or seeds (Snow and Palma 1997). The risk from genetically modified (GM) plants results from the possibility of gene contamination producing adverse effects on biological diversity by introducing herbicide or insect resistance into related plants or weeds (NAS 2002). The concern about the leakage of genes from GM plants into the environment has primarily focused on pollen that could be wind-borne for long distances (Paul et al. 1995, Timmons et al. 1995).

During the period of risk assessment in Japan, physical containment is applied as a measure of reducing gene flow via the dispersal of pollen from GM plants into the surrounding environment. The first step of risk assessment is to confirm whether introduced genes in individual plantlets can be safely and stably enclosed to maintain stable environmental conditions, such as a constant air temperature, light intensity, and supply of water and nutrients. After this is confirmed, the GM plants are moved into a greenhouse where all the openings are covered by 1-mm fine mesh. Under variable environmental conditions in the special screened greenhouse, the safety and stability of the translated gene traits is reconfirmed in the second step. For the third step, the GM plants are cultivated in an insulated experimental field for a final risk assessment.

The focus of this study involved the second step, i.e., the special screened greenhouse, which represents the first substantial risk of gene flow from GM plants into the environment during the risk assessment period. To estimate the mesh size that is capable of effectively reducing pollen dispersal, we examined the pollen grain sizes of plants used as materials for transgenic experiments in Japan. Second, we researched whether thrips were capable of transporting pollen grains as an indicator of the potential of small insects, other than general pollinators, to function as pollen vectors. Because thrips are very common small insects observed on many kind of crop flowers and leaves as vermin and

the body length are usually around 1 mm, it might be a candidate as pollen vector that could pass through 1 mm mesh. Reports about thrip pollination do exist (Ananthakrishnan 1993; Mound and Terry 2001; Sakai 2001). Finally, the relationship between the mesh size and air temperature of the covered space as a model of a greenhouse was investigated to estimate the extent to which mesh size could be minimized.

The actual amount of gene flow from GM plants and the associated consequences depends on the plant, transgene, trait encoded, particular environment, and risk management practices (Glover 2002). Therefore, case-by-case studies are needed to adequately assess the likelihood and potential impact of gene flow. However, conducting case-by-case research is difficult from a practical perspective. In this paper, we offer basic research data to advance the applied studies of GM plant materials.

Materials and methods

Determining pollen grain sizes

We conducted a literature review to evaluate the pollen sizes of the major plants used as materials for transgenic studies in Japan (Table 1). Plant species that had not been evaluated for pollen grain size in the literature were collected, and their pollen grains were measured (Table 1).

Pollen was collected on glass slides by shaking the anthers of one flower per plant. This treatment was performed for three to five flowers from each plant species. With coherent pollen, such as those of insect-pollinated plants, 10–20 μl of xylene was added to distribute the pollen equally on the glass slide.

Digital images of the pollen were photographed under a Leica DMR microscope at 50 or 100× magnification. Digital images of 1-micrometer-long glass were also photographed at the same magnification ratio as that of the pollen images to convert the pollen grain size data from pixels into micrometers. ImageJ for the Macintosh OSX (Apple, Cupertino, CA), a public domain image processing and analysis program developed at the National Institutes of Health (NIH), was used to measure the size of pollen from the digital image data (detailed method submitting).

The color images of pollen were converted to black-and-white images (two-valued data), and the threshold level was determined automatically by analyzing the histogram of the entire image on ImageJ. A particle-fit ellipse function was used to find and measure the primary (major) and secondary (minor) axes of the best fitting ellipse to each pollen image. After removing the end values, such as dust, the measured data were analyzed to calculate the means of the major and minor lengths of pollen in micrometers.

Small insects as pollen vectors

Images of thrips were collected from strawberry flowers cultivated in a test field of the Gene Research Center in the University of Tsukuba to estimate the mesh size through which pollen-eating insects could pass. Thrips were shaken off the strawberry flowers and onto a plastic dish by agitating the flowers with tweezers. Twenty thrips that were more than 0.5 mm in body length were inserted into an open-topped plastic box (W : D : H = 10 : 10 : 50 cm) with different sized meshes (from the upper layer, 400, 200, 100, and 50 μm) drawn inside horizontally (Figure 1A). After the open top was covered with a plastic cap, the box was incubated under fluorescent light (light quantum flux density, 200 μmol m^{-2} s^{-1}) at room temperature for 2 h. The number of thrips reaching each mesh layer was counted, and the same treatment was performed in triplicate.

To elucidate the possibility that small insects can transport the pollen of entomophilous flowers, wild-type chrysanthemum flowers that were cultivated in a test field of the Gene Research Center in the University of Tsukuba were sampled in October 2003 (Figure 2A). Pollen was shaken onto a plastic dish by agitating the flowers with tweezers, and thrips and a variety of mites that were on the flowers also fell into the dish. Digital images of the pollen grains from the chrysanthemums were photographed under a scanning probe microscope (NT-MDT, Tokyo Instruments, Inc., Japan; Figure 2B). Digital images of the small insects were also photographed under a Leica DMR microscope at 10× magnification (Figure 2C, D).

Influence of different mesh sizes on the temperature in a greenhouse model

We constructed small greenhouse models to investigate the occurrence and effects of rising greenhouse temperature on the risk assessment of GM plants when fine mesh $<$1 mm is used on the side windows.

Four plastic boxes (W : D : H = 30 : 30 : 30 cm) that each had four side windows (20×20 cm) and a top that was covered by Saran-wrap were constructed (Figure 3A). Each side window of each box was covered by one size of fine mesh (either 50, 100, 200, or 400 μm from front to back; Figure 3A). A small thermo sensor (Hioki Co. Ltd., Japan) was placed in the center of each box to record the temperature inside the box. The sensor was hardwired to a data logger attached to the outside wall of each box.

The small greenhouse models were set to log the temperature data and were placed on a table in a southside field in front of the Gene Research Center for 2 or 3 days a month from October 2003 to May 2004, except during times of rain or strong winds. At the same time, meteorological data, including air temperature, light intensity, and wind speed, were collected by a

Table 1. Names and pollen grain sizes of plants used for transgenic experiments in Japan.

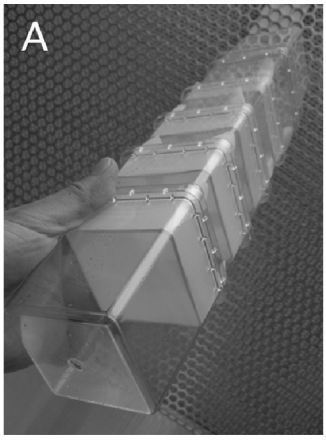
Scientific Name	Family	English Name	Pollen Grain Size[1)] mean±S.D. (μm)	Pollination Form	Research Methods
Arabidopsis thaliana L. Heynh.	Cruciferae	mouseearcress	23±2×35±3	self-pollination	measured
Brassica rapa L.var. amplexicaulis	Cruciferae	rape	22±2×41±2	insect-pollination	measured
Capsicum annuum L.	Solanaceae	red pepper	24±3×43±6	insect-pollination	measured
Capsicum annuum L. var.grossum Sendt.	Solanaceae	green pepper	26±2×48±3	insect-pollination	measured
Cucumis melo L.	Cucurbitaceae	melon	45±4×52±5	insect-pollination	measured
Cucumis sativus L.	Cucurbitaceae	cucumber	49±3×56±4	insect-pollination	measured
Cucurbita moschata L.	Cucurbitaceae	pumpkin	124±4×132±4	insect-pollination	measured
Dendranthema×grandiflorum (Ramat) Kitamura	Compositae	chrysanthemum	27±2×36±6	insect-pollination	measured
Dianthus caryophyllus L.	Caryophyllaceae	carnation	49±8×56±8	insect-pollination	measured
Eustoma grandiflorum Shinn.	Gentianaceae	prairie gentian	28±3×39±6	insect-pollination	measured
Fragaria grandiflora Ehrh.	Rosaceae	strawberry	16±3×22±5	insect-pollination	measured
Gladiolus spp.	Iridaceae	gladiolus	51±12×95±18	insect-pollination	measured
Glycine max Merrill	Fabaceae	soybean	9±1×14±1	insect-pollination	measured
Hordeum vulgare L. var. distichon	Poaceae	barley	45±4×58±4	wind-pollinatiion	measured
Lactuca sativa L.	Compositae	lettuce	32±3×35±4	insect-pollination	measured
Lotus corniculatus L. var. japonicus Regel	Fabaceae	birdsfoot trefoil	16±3×24±3	insect-pollination	measured
Lycopersicon esculentum Mill. cv. Money Maker	Solanaceae	tomato	19±2×35±3	insect-pollination	measured
Nicotiana tabacum L.	Solanaceae	tobacco	12±1×21±2	insect-pollination	measured
Oryza sativa L.	Poaceae	rice plant	36±3×41±5	wind-pollinatiion	measured
Petunia×hybrida hort. Vilm.	Solanaceae	garden petunia	26±2×45±4	insect-pollination	measured
Solanum melongena L.	Solanaceae	eggplant	30±3×40±5	insect-pollination	measured
Solanum tuberosum L.	Solanaceae	potato	22±4×30±6	insect-pollination	measured
Torenia fournieri Linden	Scrophulariaceae	wishbone flower	22±4×33±7	insect-pollination	measured
Triticum aestivum (L.) Thell.	Poaceae	wheat	44±4×56±5	wind-pollinatiion	measured
Zea mays L.	Poaceae	corn	78±5×95±5	wind-pollinatiion	measured
Antirrhinum majus L.	Scrophulariaceae	common snapdragon	21×20–21	insect-pollination	ref.[2)]
Cajanus cajan L.	Fabaceae	pigeonpea	34–40×35–43	insect-pollination	ref.[3)]
Camellia japonica L.	Theacea	camellia	32–37×38–43	insect-pollination	ref.[3)]
Carica papaya L.	Caricaceae	papaya	28–30×28–30	insect-pollination	ref.[2)]
Carthamus tinctorius L.	Compositae	safflower	43–45×43–45	insect-pollination	ref.[2)]
Cryptomeria japonica (L. fil.) D. Don cv. Sekkansugi	Taxodiaceae	ceder	30–33×34–39	wind-pollinatiion	ref.[2)]
Gossypium hirsutum L.	Malvaceae	cotton	110–130×110–130	insect-pollination	ref.[2)]
Ipomoea batatas L. var. edulis	Convolvulaceae	sweet potato	94–100×94–100	insect-pollination	ref.[2)]
Ipomoea nil (L.) Roth	Convolvulaceae	morning glory	119–130×119–130	insect-pollination	ref.[2)]
Malus pumila Miller var.domestica Schneid.	Rosaceae	apple	27–28×30–33	insect-pollination	ref.[2)]
Oenanthe javanica DC.	Apiaceae	Japanese parsley	16×25.5–27	insect-pollination	ref.[2)]
Phaseolus vulgaris L.	Fabaceae	French bean	37–46×40–55	insect-pollination	ref.[2)]
Raphiolepis umbellata Makino	Rosaceae	yeddo-hawthorn	27–28.5×31–32.5	insect-pollination	ref.[2)]
Scutellaria baikalensis Georgi	Lamiaceae	Baikal skullcup	15.5×15.5	insect-pollination	ref.[2)]
Vigna angularis (Wild.) Ohwi et Ohash	Fabaceae	adzuki bean	37.5–40.5×41–41.5	insect-pollination	ref.[2)]
Zoysia japonica Steud	Poaceae	Japanese lawngrass	35–37×35–37	wind-pollinatiion	ref.[2)]
Atropa belladonna L.	Solanaceae	belladonna	No data	—	—
Beta vulgaris L. var. rapa	Chenopodiaceae	sugar beet	No data	—	—
Brassica campestris L. var. amplexicaulis	Cruciferae	Chinese Cabbage	No data	—	—
Brassica oleracea L.	Cruciferae	cauliflower	No data	—	—
Brassica oleracea L.	Cruciferae	broccoli	No data	—	—
Delphinium L.	Ranunculaceae	larkspur	No data	—	—
Medicago sativa L.	Fabaceae	alfalfa	No data	—	—
Panicum miliaceum L.	Poaceae	millet	No data	—	—
Paulownia fortunei Hemsl.	Scrophulariaceae	paulownia	No data	—	—
Populus nigra L. var. italica (Duroi) Koehne	Salicaceae	poplar	No data	—	—
Pyrus communis L.	Rosaceae	pear	No data	—	—
Senecio cruentus DC.	Compositae	cineraria	No data	—	—
Vitis vinifera L.	Vitaceae	European grape	No data	—	—

The pollen grain sizes of 25 species were measured directly, and the pollen grain sizes of 16 other species were described in the previous literature.

[1)] The pollen grain sizes directly measured are described by the minor axis length of the best fitting ellipse±S.D.×the major axis length of the best fitting ellipse±S.D. (n=50−approximately 200). The pollen grain sizes in the previous literature are described by diameter×diameter.

[2)] Ikuse 1956.

[3)] Huang 1972.



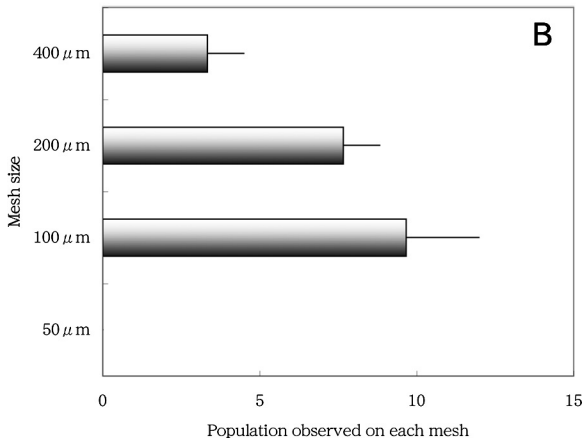


Figure 1. Determining whether thrip can transit fine mesh. (A) Differing sizes of fine mesh (from the upper layer: 400, 200, 100, and 50 μm) were drawn horizontally inside a plastic box to determine thrip transit abilities. (B) The results of the thrip transit experiments. The number of thrips reaching each mesh was counted, and the same treatment was performed in triplicate. Thrips were not found on the 50-μm mesh layer.

weather station equipped with a Vantage Pro (Davis Instruments Co., Hayward, CA; Figure 3A). All data were taken at 5-min intervals.

The difference in air temperature between the outside and inside of each box was calculated by subtracting the air temperature values outside the box from the values inside the box for each recorded point to discern the effect of various air temperatures.

Results and discussion

Pollen grain size

We found 54 species that were listed as major plant samples used for gene recombination experiments in Japan. We directly measured the pollen grain sizes of 25 species and found the pollen grain sizes of 16 other species in the literature (Table 1). In our list, the smallest pollen grains were approximately 9–22 μm in diameter and were from a group of plants that included *Fragaria*

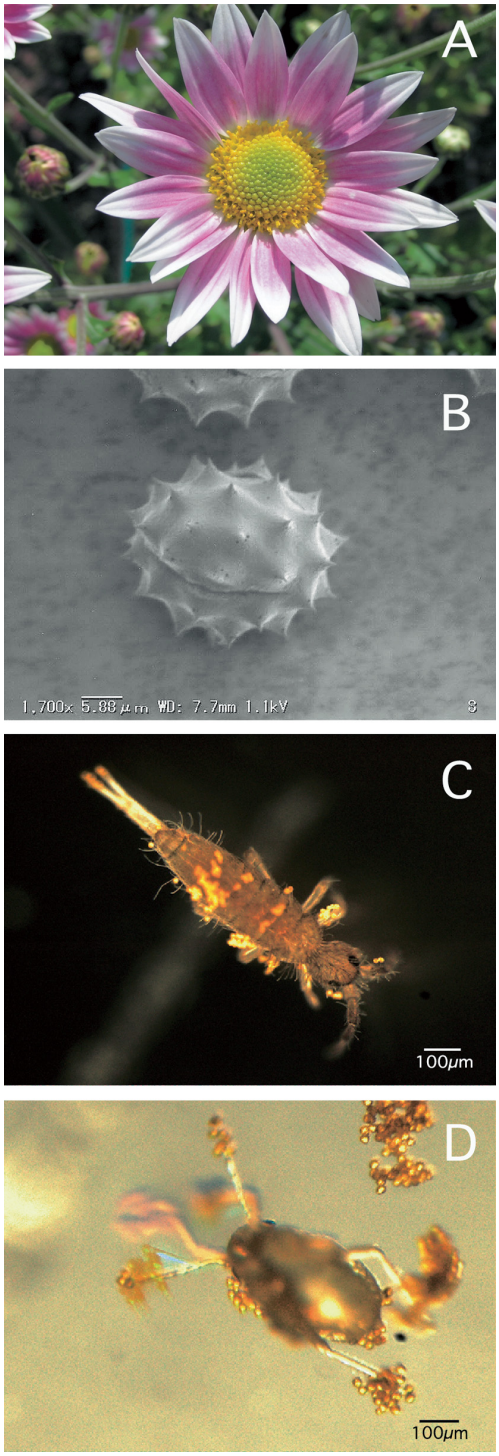


Figure 2. The potential of small insects to be unusual pollen vectors. (A) Wild-type flowers of chrysanthemums cultivated in a test field of the Gene Research Center at the University of Tsukuba were sampled to collect small insects. (B) Digital images of chrysanthemum pollen grains were photographed under a scanning probe microscope. The diameter was approximately 20–22 μm. (C) Image of a thrip whose body length was approximately 1 mm with a pollen grain. (D) Image of a mite whose body length was approximately 500 μm with a pollen grain.

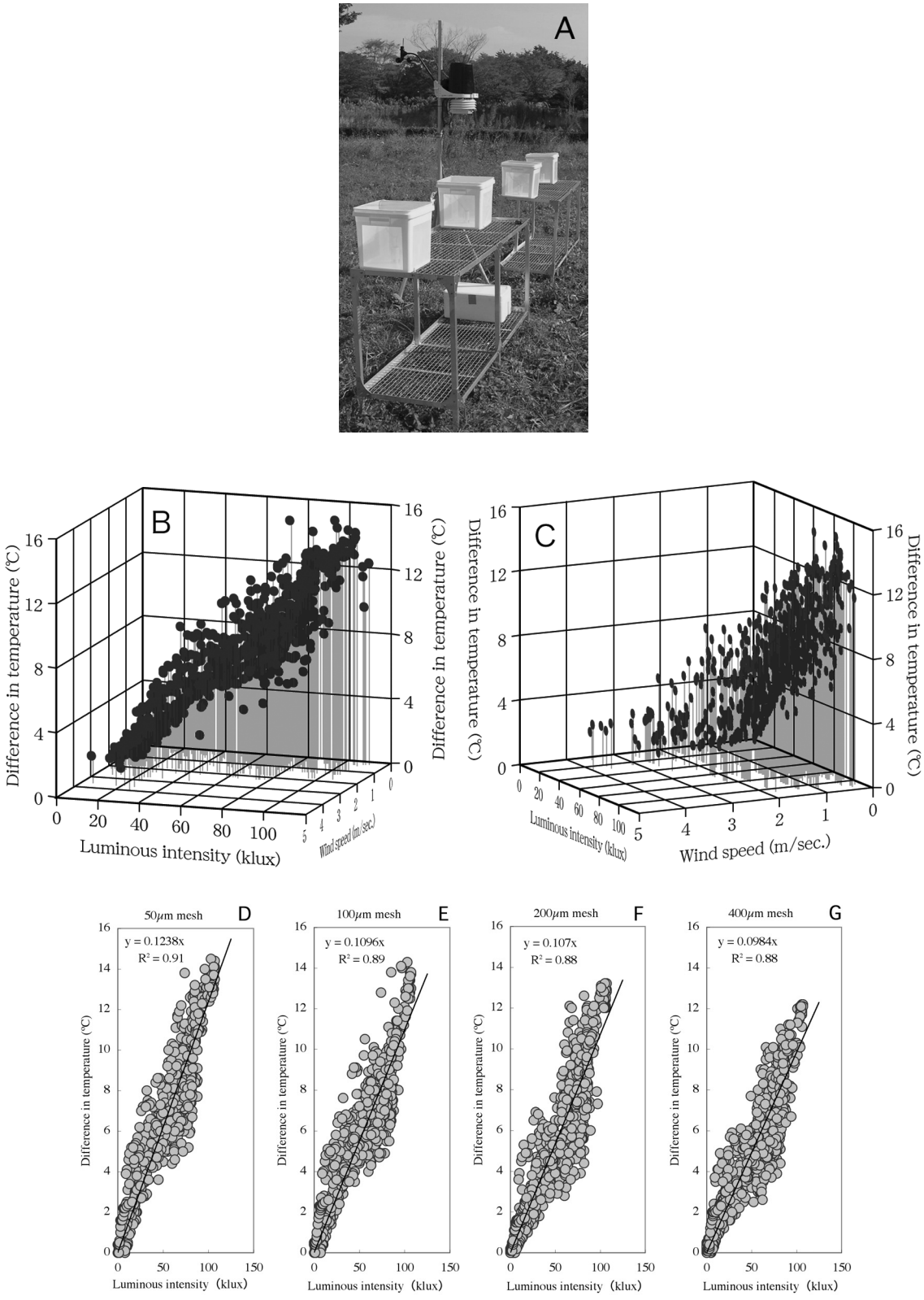


Figure 3. The influence of different mesh sizes on the temperature inside the greenhouse models. (A) Meteorological data were collected by a Vantage Pro weather station (center of Figure 3A). Small greenhouse model boxes, each with a single size of mesh covering four side windows (50, 100, 200, or 400 μm from front to back) and tops covered by Saran-wrap, were used to measure the temperatures inside the boxes attributable to the different mesh sizes. (B, C) The air temperature difference between the outside and inside of each box was calculated by subtracting the outside air temperature values from the inside values for every recorded point to clarify the effect on air temperature. The calculated temperature values of a box with 50-μm mesh were plotted on 3-D graphs with wind-speed values (m/s) on the *x*-axis and luminous intensity values (klux) on the *y*-axis. Figure 3B shows a view from the *x*-axis and C is from a view of the *y*-axis. (D–G) The calculated temperature values of the boxes with 50, 100, 200, and 400-μm mesh were plotted on graphs D–G, respectively, with luminous intensity values (klux) on the x-axis, except data over 1.0 m/s in wind speed.

grandiflora Ehrh, *Nicotiana tabacum* L., and *Glycine max*. The largest pollen grains were approximately 110–130 μm in diameter and were from a group of plants that included *Cucurbita moschata* L., *Gossypium hirsutum* L., and *Ipomoea nil* (L.) Roth in our list. These results indicated that the pollen we researched could pass through the 1-mm mesh of special screened greenhouses.

There was no relationship between the pollen grain size and the pollination type. Several species on our list were wind-pollinated outcrossing species (Table 1). There is evidence of wind being the pollination vector in certain species, but many species do not depend on the random pollination pathways offered by wind because wind is only useful in situations in which large populations of a very limited number of species are present. Actually, many wind-pollinated outcrossing species are gregarious plants. Most of the GM plants species on our list were insect-pollinated outcrossing species (Table 1).

In the case of *Glycine max*, which had the smallest pollen grain, and even though natural cross-pollination is dependent upon the distance between plants, genotypes involved, environment, and insects present (Weber and Fehr 1967), insects likely have the biggest impact on natural cross-pollination (Ray et al. 2003). In most insect-pollinated outcrossing species, highly viscous nectar in the flower may help the pollen attach to the bodies of insects and thus reduce the amount of pollen grain dispersal by wind. In *G. max*, the natural cross-pollination rate in adjacent rows in a field experiment was about 0.04% (Woodworth 1922), whereas in another field experiment, the natural crossing rate for plants equally spaced 15.2 cm apart averaged 1.8% (Ray et al. 2003). However, the crossing rates of wind-pollinated plants are much higher than insect-pollinated plants. In the case of *Zea mays*, the average outcrossing rate in adjacent rows under field conditions was approximately 20% (Jones and Brooks 1950). From these reports, when cultivated in a greenhouse during a risk assessment, GM plants that are noted to be wind-pollinated outcrossing species unless invaded by a pollinator have the potential to transport pollen grains for long distances.

Small insects as possible transporters of pollen grains

Multiple images of thrips were taken from each layer with 100, 200, and 400-μm mesh (Figure 1B), and thrips with body lengths over 0.5 mm were unable to pass through the 100-μm mesh (Figure 1B).

Chrysanthemum pollen grains were found attached to the body surfaces of mobile thrips and mites (Figure 1C, D). As suggested by Chiari et al. (2005), rich nectar in the flowers may have made it easier for the pollen grains to come in contact with the body surfaces of these insects.

Our results indicated the possibility that small insects, which could pass through 1-mm mesh, might play a role as pollination vectors for insect-pollinated outcrossing plants. Although this type of small insect is thought to usually move in a narrow range compared to normal pollinators such as bees or butterflies, to be thorough, investigators should note any insect-pollinated outcrossing plant species with nectar that has a higher viscosity than wind-pollinated flowers and should adequately use insecticide in greenhouses during a period of risk assessment. In this research, the use of fine mesh $<$100 μm was effective in preventing small-insect transport of pollen. However, the balance between preventing insects from invading the greenhouse and increasing the inside air temperature must be considered.

Influence of window mesh size on the temperature in the greenhouse model

An increase in the luminous intensity produced an increase in the difference between the air temperature outside and inside of each box (Figure 3B). The difference in air temperature between the outside and inside of each box also increased with a decrease in the wind speed (Figure 3C). Linear relationships were found between the difference in air temperature and both the luminous intensity and wind speed, which indicated that the degree of temperature elevation in the greenhouse depends on both factors, luminous intensity and wind speed. The inclination of the linear regression curve was larger in the box with the finer mesh, indicating that the air temperature inside the box with the finer mesh increased more than the temperature inside the box with the loose mesh (Figure 3D, E, F, G).

The difference between outside and inside air temperatures was 10–15°C at 100 klux in boxes with $<$200-μm mesh (Figure 3D, E, F, G). In the other experiment, the air temperature difference between the outside and inside of the box with 1-mm mesh was between 6 and 9°C at 100 klux (Watanabe, unpublished data). The air temperature difference between the outside and inside of an agricultural pipe house covered by 1-mm mesh and located in a cornfield from July to August 2004 was between 5 and 8°C at 100 klux (Watanabe, unpublished data). In the present study, when the air temperature was 35°C, temperatures inside the boxes with $<$200-μm mesh were over 45°C. These conditions are too severe for most crops to avoid heat damage. The data in this study was not sufficient to estimate an accurate rate of temperature rise in the various greenhouses because the effects of other factors, such as differences in building materials, the scale of space, and the opening area versus the construction surface area, were not studied. More data are needed under varying conditions, and materials other than fine mesh should be

evaluated.

Conclusion

Verifying the safety and stability of translated gene traits in GM plants through exposure to natural conditions in special screened greenhouses is essential, despite the additional regulatory requirement to restrict gene flow as much as possible.

We propose the following actions. Characterize the pollination type and pollen characteristics of the GM plant. In the case of a wind-pollinated plant, consider the related plants or weeds around the greenhouse. Determine the critical temperatures of the GM plant samples and then specify the cultivation period to avoid heat damage. Use 400 to 1000-μm mesh if the cultivation period involves the summer season. Use a shade curtain to drop the temperature inside the greenhouse during the hot season. Practice a thorough pest control regimen in the greenhouse during the period of risk assessment.

Acknowledgments

This study was supported by a Research Grant of the Life Science Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT; Japan).

References

Ananthakrishnan TN (1993) The role of thrips in pollination. *Curr Sci* 65: 262–264

Chiari WU, Toledo VD, Ruvolo-Takasusuki MCC, Attencia VM, Costa FM, Kotaka CS, Sakaguchi ES, Magalhaes HR (2005) Floral biology and behavior of Africanized honeybees *Apis mellifera* in soybean (*Glycine max* L. Merril). *Braz Arch Biol Technol* 48: 367–378

Glover J (2002) *Gene Flow Study: Implications for the release of genetically modified crops in Australia*. Bureau of Rural Sciences, Canberra, Australia, pp. iv–vi

Huang TC (1972). *Pollen flora of Taiwan*. National Taiwan University Botany Department Press, Taipei, China, pp 142, 232

Ikuse M (1956) *Pollen Grains of Japan*. Tokyo, Hirokawa Publishing Co. Tokyo, pp 36, 41, 87, 95, 104, 108, 126, 129, 132, 149

Jones MD, Brooks JS (1950) Effectiveness of distance and border rows in preventing outcrossing in corn. *Oklahoma Agricultural Experiment Station Technical Bulletin* No. T-38, Stillwater, OK, USA, p 18

Mound LA, Terry I (2001) Thrips pollination of the central Australian cycad, *Macrozamia macdonnellii* (Cycadales). *Int J Plant Sci* 162: 147–154

NAS (National Academy of Science, USA) (2002) *Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation*. National Academic Press, Washington, DC, USA, pp 52–100

Paul EM, Thompson C, Dunwell JM (1995) Gene dispersal from genetically modified oilseed rape in the field. *Euphytica* 81: 283–289

Ray JD, Kilen TC, Abel CA, Paris RL (2003) Soybean natural cross-pollination rates under field conditions. *Environ Biosaf Res* 2: 133–138

Sakai S (2001) Thrips pollination of androdioecious *Castilla elastica* (Moraceae) in a seasonal tropical forest. *Am J Bot* 88: 1527–1534

Snow AA, Morán Palma PM (1997) Commercialization of transgenic plants: potential ecological risks. *BioScience* 47: 86–96

Timmons AM, Óbrien ET, Charters YM, Dubbels SJ, Wilkinson MJ (1995) Assessing the risks of wind pollination from fields of genetically modified *Brassica napus* ssp. *oleifera*. *Euphytica* 85: 417–423

UNEP (United Nations Environmental Program) (2004) *Cartagena Protocol on Biosafety to the Convention on Biological Diversity, 23–27 February 2004*. Kuala Lumpur, Malaysia.

Weber CR, Fehr WR (1967) Effect of hybridization and thermal neutron irradiation on quantitative characters of soybeans. *Crop Sci* 7: 78

Woodworth CM (1922) The extent of natural cross pollination in soybeans. *Agron J* 14: 278–283